Research Article

Identification of Bioactive Compounds in Acetone Leaf and Stem-Bark Extracts of *Psychotria dalzellii* Hook.f. by GC-MS Analysis and Evaluation of *in vitro* Anti-bacterial Properties

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Submission Date: 14-09-2023; Revision Date: 21-10-2023; Accepted Date: 02-11-2023.

ABSTRACT

Background: *Psychotria dalzellii* Hook.f. (Rubiaceae) is an ethnobotanically important plant species, traditionally the stem part is used as a remedy for pruritus and the leaf juice is utilized in the preparation of eye drops for migraines. However, these claims lack of scientific validation. The current study focused on Gas Chromatography-Mass Spectrometry analysis and *in vitro* evaluation of antibacterial properties of leaf and stem bark acetone extracts of *P. dalzellii.* **Materials and Methods:** *P. dalzellii* leaf and stem-bark samples were collected from Joida, Western Ghats, Karnataka, India. The collected plant materials were subjected to Soxhlet extraction using acetone. The presence of phytochemicals were investigated by preliminary qualitative screening and GC-MS for quantitative estimation. Then, the antibacterial potential was analysed by agar well diffusion technique against selected plant and animal pathogenic bacterial strains. **Results:** GC-MS chromatogram of acetone stem-bark extract shows the presence of 15 chemical constituents in that, Resorcinol (58.36%) and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (8.81%) were found to be the major phytoconstituents. Whereas, the leaf extract shows 19 bioactive compounds in that, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (26.99%); Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (21.99%) and Resorcinol (17.87%) were found to be the major compounds. *P. dalzellii* extracts of stem-bark and leaf exerted greater antibacterial activity towards all tested bacterial strains. Among leaf and stembark extracts, the stembark acetone extract were shown to be the most potent against Gram-negative pathogenic bacterial strains like *Pseudomonas syringae* (19.33 ± 0.16 mm), *Klebsiella pneumoniae* (19.25 ± 0.14 mm), *Xanthomonas compestris* (18.16 ± 0.16 mm) and *Escherichia coli* (17.25 ± 0.14 mm). **Conclusion:** This study provides new insights into the *Psychotria dalzellii* as a potential tool for antibacterial drug discovery through *in vitro* investigation that could be used to treat human and plant infectious diseases.

Keywords: *Psychotria dalzellii,* Phytochemical analysis, GC-MS, Anti-bacterial.

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INTRODUCTION

The world is still suffering tremendously from life-threatening infectious diseases brought on by bacteria, viruses, fungi and other pathogens.[1] It is possible to control these pathogenic bacteria using a variety of antibiotics that can either kill the bacteria's or stop their growth. However, the problem is extensive, irregular, inappropriate and indiscriminate use of antibiotics has resulted in the emergence of antibacterial resistance.[2] Where the bacteria can develop mechanisms to protect themselves against aggressiveness from the environment such as the natural environment, competing bacteria, host defence or antibiotics by changing the anti-infective molecule's mode of action or by producing enzymes that break down the infecting agent.[3] According to the Centers for Disease Control and Prevention, every year

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DOI: 10.5530/ajbls.2023.12.66

in the United States at least 2 million people encounter a drug-resistant bacterial infection, which results in at least 23,000 deaths.[4] Thus, drug resistance has emerged as a major issue recently in healthcare. Therefore, it is critical to generate novel medications using natural plants in order to combat bacterial resistance.[3]

The plant possesses different types of secondary metabolites to defend against stress and pathogenic infection. They are the complementary source of antibacterials that are safer, natural, more affordable, and time-tested source than other antibiotics.[5,6] The finding of active compounds from natural sources is the initial step in the development of new therapeutics.[7] For this, scientists are more interested in investigating secondary metabolites derived from plants to create novel therapeutic medications. In accordance with the World Health Report on Infectious Diseases (2000), combating bacterial resistance is one of the most significant problems for WHO in the current era. As a result, during the past 10 years, there has been an increase in research on the uses of plants to cure various disorders.[8]

In an effort to broaden the range of antibacterial compounds derived from natural resources, *Psychotria dalzellii* Hook.f belonging to the Rubiaceae family has been selected. In the Indian literature, this plant has been traditionally described to be useful to treat pruritus and migraine headaches.[9,10] Besides this, Abhishek *et al.* 2019 reported that this plant extract has antidiabetic and antioxidant properties.[11] Some other *Psychotria* species of this genus, such as *P. gardineri* (leaves and branches), *P. microlabastra* (stem, leaves and root bark) and *P.nigra* (branches and leaves) have been the subject of previous pharmacological studies, which have demonstrated their extracts have antibacterial properties.[3] However, the antibacterial properties of this plant have not been examined. Hence, the present attempt was focused on identifying various bioactive components and assessing the antibacterial potential from acetone leaf and stembark extracts of *P. dalzellii*.

MATERIALS AND METHODS

Extraction of bioactive compounds

Fresh plant materials like leaf and stem bark of *P. dalzellii* were collected from Joida, Western Ghats of Karnataka, India, and a voucher specimen (KU/AB/RN/TAB-01) of the plant was deposited in the Department of Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga. The collected samples were rinsed with running water, allowed for shade drying and then made into powder with the help of a mill blender. The powdered sample was subjected to the Soxhlet extraction. After the extraction process was finished, the obtained extracts were air-dried and stored in air-tight vials at 4ºC.

Preliminary screening of phytochemicals.

The crude leaf and stem-bark acetone extracts of *P. dalzellii* were tested to identify various bioactive constituents, which include alkaloids, glycosides, phenols, tannins, terpenoids, steroids and flavonoids using standard protocols [12,13] and each of the tests was qualitatively expressed as negative (-) for the absence and positive (+) for the presence of phytochemicals.

GC-MS profiling

The quantitative screening of bioactive constituents in acetone extracts of both leaf and stembark part of *P. dalzellii* was analysed using Gas Chromatography (Agilent 7890 series) equipped with HP-5MS column (length 30 mm; internal diameter 0.34 mm; film thickness 0.25 m). Mass Spectrometer configured to a temperature 35ºC-280ºC with a hold period of 3 min with the rate of 8 mL C/ min. The parameters for chromatography were column flow rate of 1 mL/min, injection mode: split and carrier gas was Helium 99.99%. The bioactive constituents were identified by GC-MS spectra with mass library search (NIST and AMDIS software) with their relative retention indices.

In vitro antibacterial screening

In vitro antibacterial potential of acetone leaf and stem-bark extracts of *P. dalzellii* were carried out using agar well diffusion methods[14] and screened against nine pathogenic bacterial isolates obtained from MTCC, IMTECH Chandigarh, India.

Test microorganisms

The test bacterial strains used for the antibacterial screening were *Escherichia coli* (MTCC-1599), *Xanthomonas compestris* (MTTCC-2286), *Klebsiella pneumoniae* (MTCC-7028), *Salmonella typhi* (MTCC-734), *Salmonella enterica* (MTCC-3231), *Pseudomonas syringae* (MTCC-1604), *Pseudomonas aeruginosa* (MTCC-1934) are were Gram-negative pathogens and *Staphylococcus aureus* (MTCC- 903) and *Enterococcus faecalis* (MTCC- 439) were Gram-positive pathogens.

Preparation of bacterial inoculum

The most significant distinction between nutrient media and nutrient broth is that the nutrient media has agar powder, a solidifying ingredient that makes the medium solid at room temperature. Whereas, the nutrient broth continues to be in liquid form. Usually, the medium

used to culture the inoculums is the same media used for the assay. A single colony was chosen from the plate, transferred to 100 mL sterile nutrient broth and incubated overnight at 37ºC.

Preparation of plant sample

In two separate Eppendorf tubes, 10 mg of the crude acetone extracts of *P. dalzellii* leaf and stem bark were weighed. Each extract was then dissolved in 1000 µL of DMSO. Further, it was diluted to 100, 50 and 25% concentrations.

Agar well diffusion method

The medium and the Petri plates were sterilized in an autoclave for 15 min at 121ºC and 12 lbs pressure. Further, the sterile medium was aseptically transferred in an amount of 20-25 mL to the sterile petri plates. Then the plates were allowed to solidify inside the laminar airflow chamber. 15 µL of the fresh bacterial strains were poured using a micropipette onto sterile media and swabbed evenly to make lawn culture. The plates were labelled. Using a separate sterile cork borer, wells of 6 mm diameter were punched on solidified loaded media. 20 µL extract of desired concentrations was transferred aseptically into each well of the agar plate. Ciprofloxacin (1 mg/mL) and DMSO were employed as positive control and negative controls. The plates were inoculated and incubated at 35-37ºC for 24 hr to evaluate the zone of inhibition and the experiments were performed in triplicate to obtain average data.[15]

RESULTS

Qualitative analysis of phytoconstituents

The preliminary phytochemical analysis of the leaf and stem-bark acetone extracts of *P. dalzellii* revealed that, the glycosides were present in both the acetone leaf and stem-bark extracts. Whereas, alkaloids, phenols and flavonoids were only present in the leaf extract. Tannins and steroids were present in stembark extract and they were not found in leaf extract of the plant. Further, terpenoids were not found in both the extracts [Table 1].

Quantitative GC-MS Profiling

GC-MS chromatogram of the leaf acetone extract of *P. dalzellii* shows 23 peaks corresponding to the presence of 19 bioactive chemicals [Figure 1]. Meanwhile, the plant's stem-bark acetone extract depicts 20 peaks, indicating the presence of 15 bioactive chemical elements [Figure 2]. The phytoconstituents of plant leaf and stem-bark extracts are listed in Table 2 and Table 3 respectively, along with the compound name, Retention Time (RT), peak area percentage, Molecular Formula (MF) and Molecular Weight (MW) for each.

The identified compounds in the leaf acetone extract of the plant include, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (26.99%); Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (21.99%); Resorcinol (17.87%); Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5-(2-propenyl)-, [1R-(6-endo,7-exo,8-syn)]- (3.63%); 1,7-Dimethyl-4-(1-methylethyl) cyclodecane (3.38%); Sulfurous acid, butyl heptadecyl

Table 1: Qualitative analysis of phytoconstituents in acetone leaf and stem-bark extracts of *P. dalzellii*.

Phytochemical tests	Leaf	Stem-bark
Alkaloids	+	-
Flavonoids	+	-
Tannins	-	+
Glycosides	+	+
Phenols	+	-
Steroids	-	+
Terpenoids	-	-

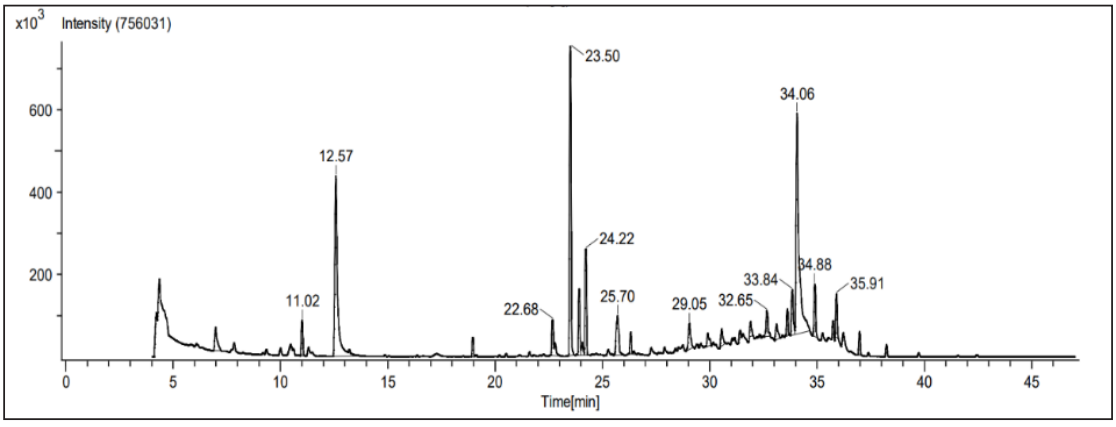

Figure 1: GC-MS chromatogram of acetone leaf extract of *P. dalzellii*.

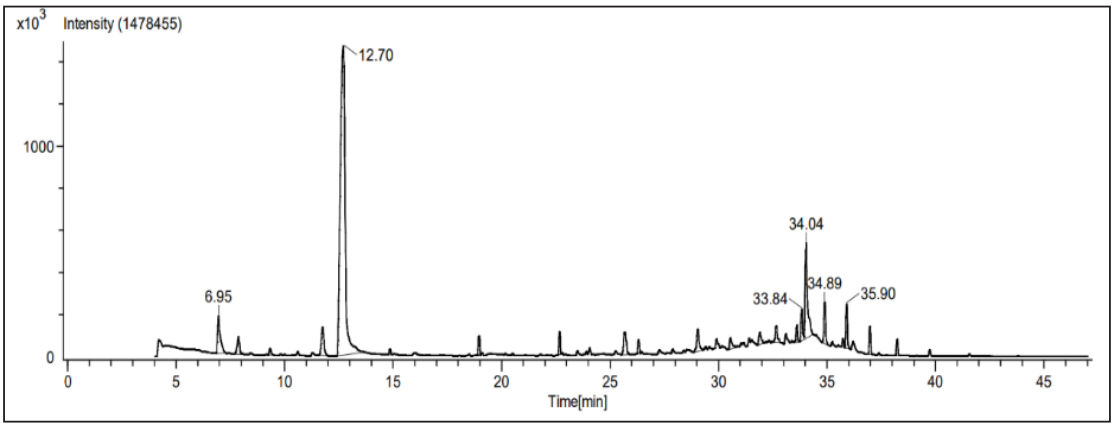

Figure 2: GC-MS chromatogram of acetone stem-bark extract of *P. dalzellii.*

Table 2: List of identified phytochemicals in crude acetone leaf extract of *P. dalzellii.*

Peak No	Retention time in min	Average % of peak area	Chemical compounds present	Molecular formula	Molecular weight
1	6.98	1.94	2-Pyrrolidinone, 1-methyl	C_5H_9NO	99
2	11.02	1.97	Benzaldehyde, 4-methyl	C_8H_8O	120
3	12.57	17.87	Resorcinol	$C_6H_6O_2$	110
4	18.96	1.02	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	$C_{15}H_{30}$	210
5	22.68	2.85	3-Octadecene, (E)-	$C_{18}H_{36}$	252
6	23.50	17.41	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
7	23.92	3.74	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
8	24.06	0.79	Phthalic acid, dodecyl 2-isopropoxyphenyl ester	$C_{29}H_{40}O5$	468
9	24.22	5.84	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
10	25.70	3.63	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5-(2 -propenyl)-, [1R-(6-endo,7-exo,8-syn)]-	$C_{21}H_{24}O7$	388
11	26.32	1.35	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	$C_{15}H_{30}$	210
12	29.05	2.06	Octadecanoic acid, 2-oxo-, methyl ester	$C_{19}H_{36}O_3$	312
13	29.91	1.01	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	$C_{15}H_{30}$	210
14	30.55	1.30	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	334
15	31.90	0.86	1-Nonene, 4,6,8-trimethyl	$C_{12}H_{24}$	168
16	32.65	1.79	Sulfurous acid, hexyl pentadecyl ester	$C_{21}H_{44}O_3S$	376
17	33.11	1.19	Sulfurous acid, butyl nonyl ester	$C_{13}H_{28}O_3S$	264
18	33.62	1.54	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	$C_{22}H_{32}F_2O_4$	398
19	33.84	2.91	2-Bromotetradecane	$C_{14}H_{29}Br$	276
20	34.06	21.99	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
21	34.88	3.15	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	376
22	35.91	2.39	Heneicosane	$C_{21}H_{44}$	296
23	36.97	1.03	Heptacosane	$C_{27}H_{56}$	380

ester (3.15%); 2-Bromotetradecane (2.91%); 3-Octadecene, (E)- (2.85%); Heneicosane (2.39%); Octadecanoic acid, 2-oxo-, methyl ester (2.06%); Benzaldehyde, 4-methyl (1.97%); 2-Pyrrolidinone, 1-methyl (1.94%); Sulfurous acid, hexyl pentadecyl ester (1.79%); Oxalic acid, 3,5-difluorophenyl tetradecyl ester (1.54%); Sulfurous acid, pentadecyl 2-propyl ester (1.30%); Sulfurous acid, butyl nonyl ester (1.19%); Heptacosane(1.03%); 1-Nonene, 4,6,8-trimethyl (0.86%) and Phthalic acid, dodecyl 2-isopropoxyphenyl ester (0.79%).

Table 3: List of identified phytochemicals in crude acetone stem-bark extract of *P. dalzellii* Hook.f.

Peak No	Retention time in min	Average % of peak area	Chemical compounds present	Molecular formula	Molecular weight
1	6.95	3.88	2-Pyrrolidinone, 1-methyl	C_5H_9NO	99
2	7.87	1.62	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	$C_{13}H_{21}NO_4$	255
3	9.33	0.49	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144
4	11.75	2.85	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126
5	12.70	58.36	Resorcinol	$C_6H_6O_2$	110
6	18.95	1.22	1-Tridecene	$C_{13}H_{26}$	182
7	22.68	1.51	3-Octadecene, (E)-	$C_{18}H_{36}$	252
8	25.65	2.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
9	26.31	0.83	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	$C_{15}H_{30}$	210
10	29.04	2.13	17-Pentatriacontene	$C_{35}H_{70}$	490
11	30.55	1.24	Sulfurous acid, octadecyl 2-propyl ester	$C_{21}H_{44}O_3S$	376
12	31.90	1.11	Sulfurous acid, octadecyl 2-propyl ester	$C_{21}H_{44}O_3S$	376
13	32.65	1.39	Sulfurous acid, octadecyl 2-propyl ester	$C_{21}H_{44}O_3S$	376
14	33.61	1.16	Oxalic acid, dodecyl 3,5-difluorophenyl ester	$C_{20}H_{28}F_2O_4$	370
15	33.84	1.84	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	376
16	34.04	8.81	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
17	34.89	2.90	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	376
18	35.90	2.95	Heneicosane	$C_{21}H_{44}$	296
19	36.98	1.78	Heneicosane	$C_{21}H_{44}$	296
20	38.24	1.05	Heptacosane	$C_{27}H_{56}$	380

Likewise, in stem bark acetone extract of the plant revealed that, Resorcinol (58.36%) was the major component followed by Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (8.81%); Heneicosane (4.73%); Sulfurous acid, butyl heptadecyl ester (4.74%); 2-Pyrrolidinone, 1-methyl (3.88%); Sulfurous acid, octadecyl 2-propyl ester (3.74%); 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (2.85%); n-Hexadecanoic acid (2.53%); 17-Pentatriacontene (2.13%); D-Alanine, N-propargyloxycarbonyl-, isohexyl ester (1.62%); 3-Octadecene, (E)-(1.51%); 1-Tridecene (1.22%); Oxalic acid, dodecyl 3,5-difluorophenyl ester (1.16%); Heptacosane (1.05%); 1,7-Dimethyl-4-(1-methylethyl) cyclodecane (0.83%) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (0.49%).

Antibacterial assay

The antibacterial activities of acetone extracts of *P. dalzellii* leaf and stem-bark material showed appreciable anti-bacterial activity against selected pathogenic strains. The results of antibacterial analysis where the inhibition zone exhibited against the selected bacteria depends on the concentration of the plant extract such as 25, 50 and 100% and they were expressed as mean ± standard error of the mean. The zone of inhibition is measured in mm. However, greater antibacterial activities are observed in stem-bark extract as compared to leaf extract of the plant at higher concentration (100%) and it showed the highest zone of inhibition against Gram-negative bacterial pathogens compared to Gram-positive, as follows *P. syringae* (19.33±0.16 mm), *K. pneumoniae* (19.25±0.14 mm), *X. compestris* (18.16±0.16 mm), *E. coli* (17.25±0.14 mm), *P. aeruginosa* (16.16±0.16 mm), *S. enterica* (16.00±0.14 mm) and *E. faecalis* (16.00±0.0 mm), *S. typhi* (14.16±0.16 mm) and *S. aureus* (14.16±0.16 mm) [Table 4, Figures 3 and 4].

While, the leaf acetone extract showed highest inhibition zone against both Gram-negetive and Gram-positive bacterial strains like *K. pneumoniae* (12.25±0.14 mm) followed by *X. compestris* (10.33±0.16 mm), *P. syringae* (9.33±0.16 mm), *S. typhi* (8.41±0.22 mm), *S. aureus* (8.25±0.14 mm), *E. faecalis* (8.16±0.16 mm) and *S. enterica* (6.33±0.16 mm). Whereas, the least inhibition of zone was revealed against *P. aeruginosa* (5.83±0.16 mm) and *E. coli* (5.75±0.14 mm) and these results were compared with standard antibacterial medication Ciprofloxacin [Table 4, Figures 5 and 6].

DISCUSSION

The phytochemical screening of the *Psychotria dalzellii* leaf and stem-bark acetone extracts reveals a variety of secondary metabolites including alkaloids, flavonoids, phenols, glycosides and tannins [Table 1]. These bioactive metabolites singly or in combinations may be responsible for several therapeutic actions including antibacterial activity.[16]

To assess an extract's *in vitro* antibacterial activity, agar well diffusion is the basic method which is a widely used technique.[17] The findings of the present study established that both the leaf and stem-bark acetone extracts of *P. dalzellii* plant showed appreciable antibacterial activities towards both the Gram-positive as well as Gram-negative bacterial strains. However, the stem-bark extract showed strong activity towards Gram-negative bacterial strains like *P. syringae*, *K. pneumoniae*, *X. compestris*, *E. coli*, *P. aeruginosa*, *S. enterica* when compared to *E. faecalis* and *S. aureus* [Table 4 and Figure 3]. The bacterial resistance may be attributed to the structure of their cell walls. Usually, Gram-positive bacteria appear to be more susceptible to the inhibitory effect of the plant extract than Gram-negative bacteria, due to their single-layered cell wall structure. Whereas, Gram-negative bacteria have a multi-layered and complex cell wall[18] with an effective permeability barrier composed of a thin lipopolysaccharide exterior membrane, which could limit the penetration of the plant extract. It has already been reported that Gram-negative bacterial strains are often more resistant to plant-origin antibacterial compounds and even show less impact when compared with Gram-positive bacteria.[19] However, the results of this study show that the acetone stem-bark extract of *P. dalzellii* has more antibacterial properties towards Gram-negative bacterial strains, which indicates that, it may contains compounds that can overcome the resistance mechanism of bacterial pathogens thus it might provide novel straight forward approaches against pathogenic bacteria.

The differential antibacterial properties of the leaf and stem bark of plant extracts may be due to the nature of biologically active components. These may include alkaloids, flavonoids, terpenoids, and other secondary metabolites.[15] Understanding the specific compounds

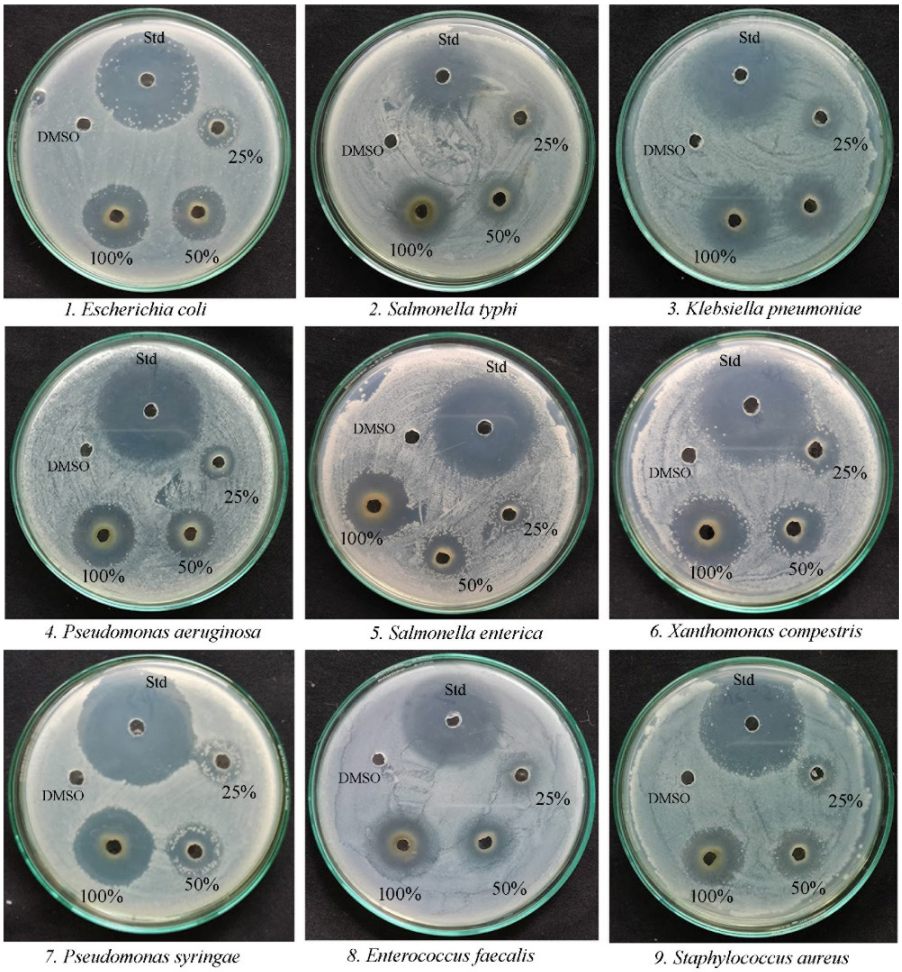

Figure 3: The antibacterial potential of the stem bark acetone extract of *P. dalzellii* against selected bacterial strains.

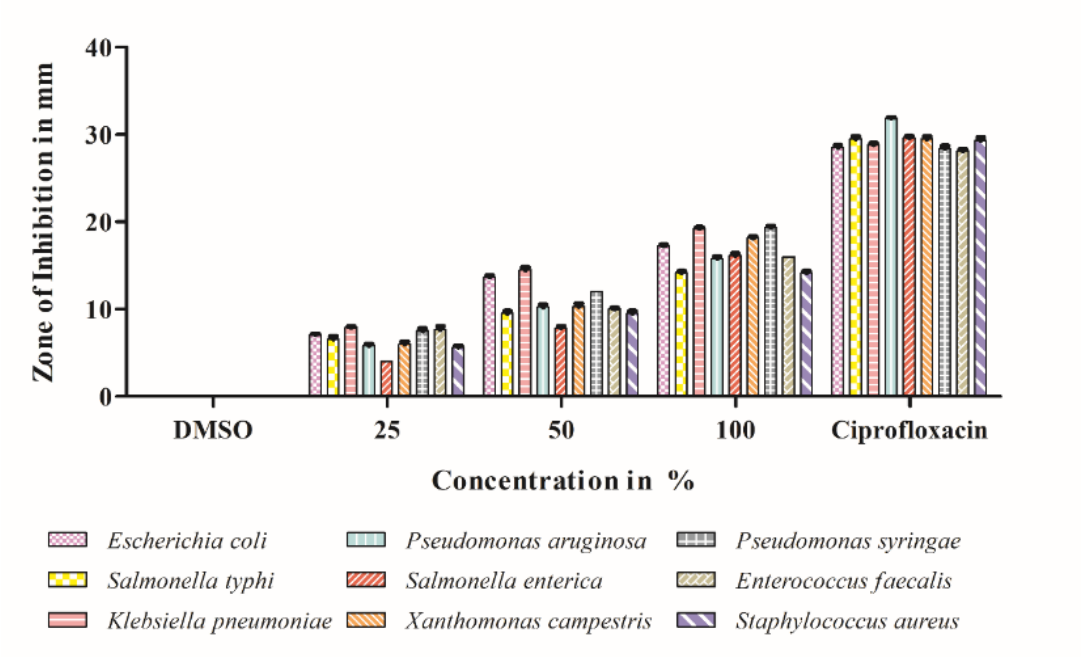

Figure 4: Antibacterial activities of the acetone stem-bark extract of *P. dalzellii* against selected bacterial strains.

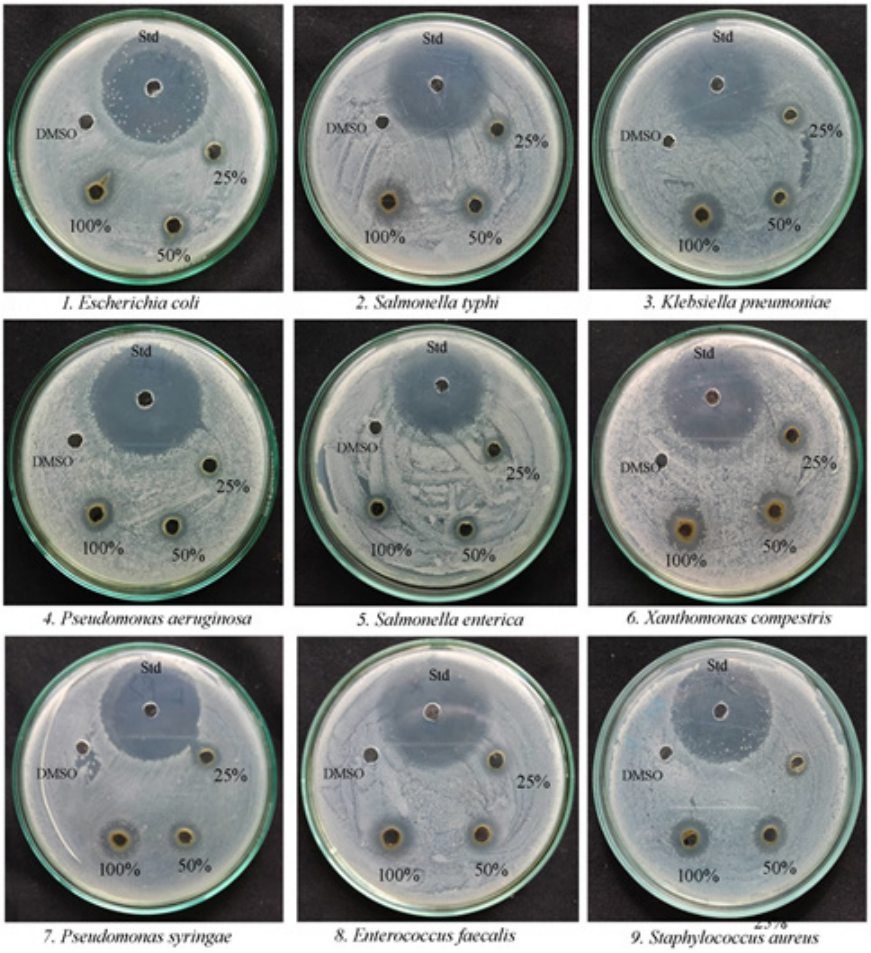

Figure 5: The antibacterial potential of the leaf acetone extract of *P. dalzellii* against selected bacterial strains.

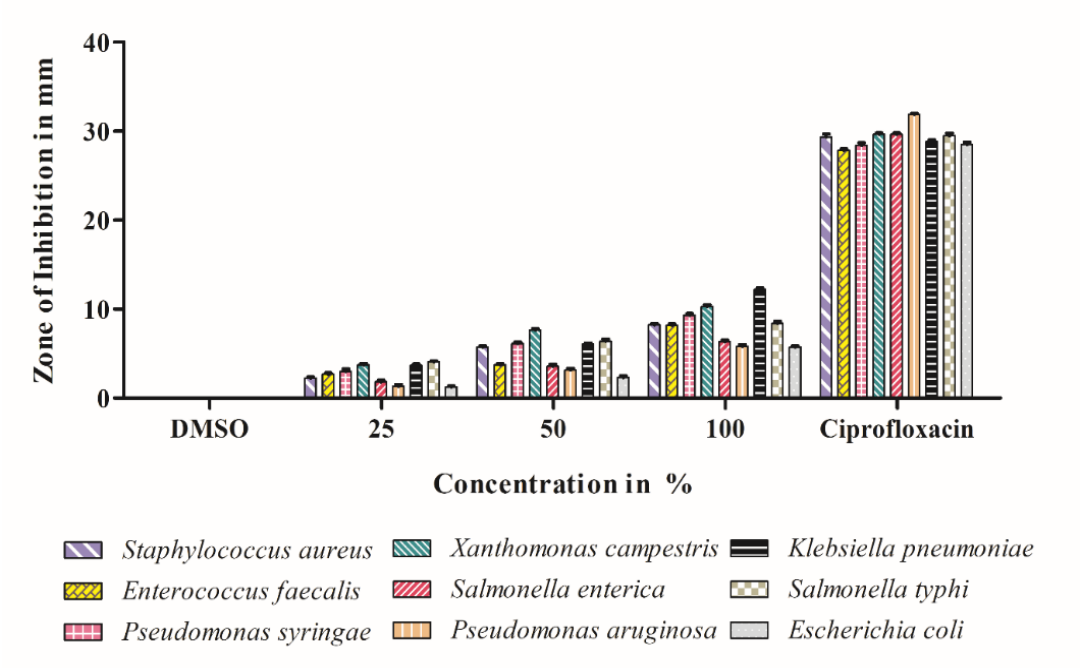

Figure 6: Antibacterial activities of the acetone leaf extract of *P. dalzellii* against selected bacterial strains.

Table 4: Antibacterial activity of leaf and stem-bark acetone extracts of *P. dalzellii* against selected bacterial pathogens.

Sl. No	Bacterial pathogens	Zone of inhibition (mm)							
		Plant leaf acetone extract			Plant stem-bark acetone extract			Standard Ciprofloxacin	Control (DMSO)
		Concentration			Concentration				
		25%	50%	100%	25%	50%	100%		
1	*Escherichia coli*	1.25±0.14	2.33±0.16	5.75±0.14	7.08±0.83	13.66±0.16	17.25±0.14	28.50±0.28	00
2	*Salmonella typhi*	4.08±0.83	6.41±0.22	8.41±0.22	6.58±0.30	9.50±0.28	14.16±0.16	29.50±0.28	00
3	*Klebsiella pneumoniae*	3.66±0.16	6.08±0.83	12.25±0.14	7.91±0.08	14.50±0.28	19.25±0.14	28.83±0.16	00
4	*Pseudomonas aeruginosa*	1.33±0.16	3.16±0.16	5.83±0.16	5.83±0.16	10.25±0.25	16.16±0.16	31.91±0.08	00
5	*Salmonella enterica*	1.83±0.16	3.58±0.22	6.33±0.16	4.00±0.0	7.83±0.16	16.00±0.14	29.66±0.16	00
6	*Xanthomonas compestris*	3.75±0.14	7.66±0.16	10.33±0.16	6.00±0.28	10.33±0.33	18.16±0.16	29.82±0.16	00
7	*Pseudomonas syringae*	3.00±0.28	6.16±0.16	9.33±0.16	7.50±0.28	12.00±0.0	19.33±0.16	28.41±0.30	00
8	*Enterococcus faecalis*	2.66±0.16	3.75±0.14	8.16±0.16	7.66±0.33	10.00±0.14	16.00±0.0	27.83±0.16	00
9	*Staphylococcus aureus*	2.25±0.14	5.75±0.14	8.25±0.14	5.66±0.16	9.50±0.28	14.16±0.16	29.33±0.33	00

responsible for antibacterial effects can provide valuable insights into potential applications in medicine and pharmaceuticals. Tannins can exert their effects by inhibiting the activity of microbial adhesins, enzymes and cellular membrane transport proteins. Flavonoids inhibit energy consumption and nucleic acid production by altering bacterial cell membranes.[20] Phenols, alkaloids and terpenoids by rupturing bacterial cell membranes or limiting DNA production.[21]

Observation of the antibacterial properties in leaf and stem-bark acetone extracts inspired the authors to examine crude extracts for chemical screening using

GC-MS; it is one of the most effective and widely used methods for separating biomolecules. Hence, the present study was carried out to determine the phytochemical constituents present in the acetone leaf and stem-bark extracts of *P. dalzellii* by Gas Chromatography and Mass Spectroscopy.

The GC-MS Chromatogram of acetone leaf extract revealed the presence of 19 bioactive compounds and stem-bark acetone extract of the plant displayed 15 compounds. The results showed that both the leaf and stem-bark extracts have seven similar compounds and the remaining compounds are different from each other [Tables 2 and 3]. Among them, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol was the major compound in leaf extract associated with anticancer and antibacterial properties.[22] Compound Resorcinol was the major compound present in both stem-bark and leaf extracts which shows antibacterial and antioxidant activities[23] followed by compound 3-Octadecene, (E) is known to possess antibacterial, antioxidant, and anticancer properties.[24] Compound Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester was reported to possess antimicrobial properties.[25] Compound Sulfurous acid, butyl heptadecyl ester are used as an acidifier and arachidonic acid inhibitors. Additionally, it also increases aromatic amino acid decarboxylase activity.[26] Compound Heneicosane was reported to have antimicrobial properties.[27] The compound Sulfurous acid, octadecyl 2-propyl ester reported to have antibacterial properties.[28] The compound 2-Furancarboxaldehyde, 5-(hydroxymethyl)- has antimicrobial, antioxidant, and antiproliferative properties and it is majorly used as a preservative.[29-31] Sharath and Naika reported that n-hexadecanoic acid has anti-inflammatory, antioxidant, hypocholesterolemic, nematicidal and pesticidal properties.[32] As a consequence of the present study, most of the identified phytoconstituents in both extracts had previously been reported to have antibacterial properties, which might be implicated in the antibacterial property of this plant. Additionally, several species of *Psychotria* genus have been documented to exhibit antibacterial properties.[8,33-35] The presence of a zone of inhibition confirmed the inhibitory activity of *P. dalzellii* plant extracts. The zone of inhibitions of different bacteria is illustrated in Figures 3 and 5. The clear zone around the sample in the plates shows the activity of the sample [Table 4]. The zone of inhibition increases with increasing the concentration of plant extracts in all the bacterial plates.[36] These findings evident that the acetone leaf extract of *P. dalzellii* considerably inhibits the growth of tested bacterial strains and stembark extract inhibits it significantly.

CONCLUSION

The results of the current study support the use of leaf and stembark acetone extracts as a potential antibacterial agent. However, the remarkably potent antibacterial activity was shown by the stem-bark acetone extract of *P. dalzellii* towards the Gram-negative bacterial strains usually they are resistant to plant extracts, this observation is extremely significant because, there may be a possibility of generating effective medications against multidrug-resistant pathogens with such a broad spectrum of activity. Additionally, GC-MS analysis was also done to check the presence of various bioactive phytoconstituents in both acetone leaf and stem-bark extracts of *P.dalzellii*. This study concluded that the presence of chemical constituents in plant extracts helps in the inhibition of pathogenic bacterial strains causing diseases in plants and animals.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **MTCC:** Microbial Type Culture Collection; **DMSO:** Dimethyl Sulfoxide; **NIST:** National Institute Standard and Technology; **mm:** millimeters.

ACKNOWLEDGEMENT

The authors are grateful to the Department of PG Studies and Research in Applied Botany, Kuvempu University, Jnana Sahyadri, Shankaraghatta for providing the facilities for conducting research activities and also thankful to IIT SAIF Bombay, for providing the facility for GCMS analysis.

PLANT AUTHENTICATION

Plant material was identified and authenticated by a taxonomist Dr. Shivanand S. Bhat, Department of Botany, Smt. IGGFW'S College, Sagar, Karnataka, by using Flora of Anshi and Flora of Madras. A voucher sample is deposited in the Department of Post-Graduate Studies and Research in Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka, India, with voucher specimen number (KU/AB/RN/TAB-01).

AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to be published; and agreed to be accountable

for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/ guidelines.

FUNDING

No funding received.

AVAILABILITY OF DATA AND MATERIAL

All data generated and analysed along with material are included in this research article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study does not involve experiments on animals or human subjects.

COMPETING INTERESTS

The authors declare that they have no competing interests.

CONSENT FOR PUBLICATION

Not applicable.

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Cite this article: Thejashree AB, Naika R. Identification of Bioactive Compounds in Acetone Leaf and Stem-Bark Extracts of *Psychotria dalzellii* Hook.f. by GC-MS Analysis and Evaluation of *in vitro* Anti-bacterial Properties. Asian J Biol Life Sci. 2023;12(3):499-509.